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0205593.7 **9 March 2002 (09.03.2002)** **GB**
- (71) Applicant (for all designated States except US): **THE UNIVERSITY OF NOTTINGHAM [GB/GB]**; University Park, Nottingham NG7 2RD (GB).
- (81) Designated States (national): **AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NC, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.**
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- (74) Agent: **WILKINSON, Stephen, John**; Stevens, Hewlett & Perkins, 1 St Augustine's Place, Bristol BS1 4UD (GB).
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- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 03/075654 A2

(54) Title: **TREATMENT OF SURFACES POPULATED BY BACTERIA**

(57) Abstract: A surface populated by a bacteria capable of producing a biofilm is treated beneficially by contacting it with a substance having N-acyl homoserine lactone degradant activity obtained from the secretions and/or excretions of the larval form of the green bottle fly, *Lucilia sericata*. Furthermore, a synergistic effect is achieved when the larval secretions/excretions are used in combination with an antibiotic, e.g. tetracycline. Such a substance having N-acyl homoserine lactone degradant activity obtained from *Lucilia sericata* larval secretions and/or excretions also forms part of the invention.

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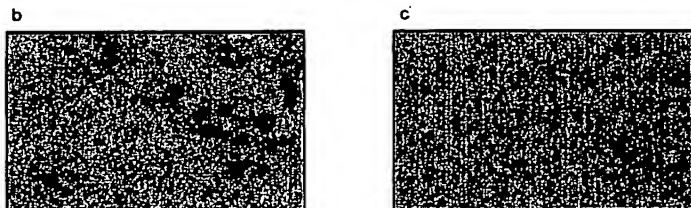
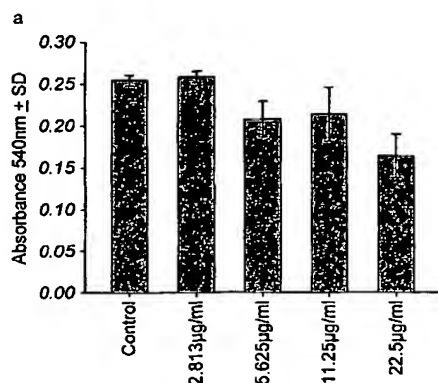
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- (71) Applicant (for all designated States except US): **THE UNIVERSITY OF NOTTINGHAM** [GB/GB]; University Park, Nottingham NG7 2RD (GB).
- (72) Inventor; and
- (75) Inventor/Applicant (for US only): **PRITCHARD, David, Idris** [GB/GB]; 83 Breach Field Road, Barrow-upon-Soar, Leicester LE12 8NN (GB).
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- (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- Published:
— with international search report

[Continued on next page]

(54) Title: TREATMENT OF SURFACES POPULATED BY BACTERIA WITH A LUCILIA SERICATA EXTRACT

Biofilm formation in the presence of *L. sericata* ES

- a) Action of *L. sericata* ES on *Staphylococcus aureus* biofilm, quantified by monitoring absorbance at 540nm of solubilised crystal violet bound to adherent cells.
- b, c) Action of ES growth of *Pseudomonas aeruginosa* biofilm on glass coverslips, visualised using BacLight™ stain b) represents control biofilm, after 20h growth.
- c) represents growth in the presence of *L. sericata* ES products (22µg/ml)

(57) Abstract: A surface populated by a bacteria capable of producing a biofilm is treated beneficially by contacting it with a substance having N-acyl homoserine lactone degradant activity obtained from the secretions and/or excretions of the larval form of the green bottle fly, *Lucilia sericata*. Furthermore, a synergistic effect is achieved when the larval secretions/excretions are used in combination with an antibiotic, e.g. tetracycline. Such a substance having N-acyl homoserine lactone degradant activity obtained from *Lucilia sericata* larval secretions and/or excretions also forms part of the invention.



(88) Date of publication of the international search report:
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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

INTERNATIONAL SEARCH REPORT

Internat Application No

PCT/03/00959

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K35/64 A61P31/00

Rec'd PCT/PTO 08 SEP 2004

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS, CHEM ABS Data, EMBASE, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	WO 03 01357 A (AVENTIS PHARMA GMBH) 20 February 2003 (2003-02-20) claims 3,19,20,23; example 2 ---	1,4, 10-13,17
X	EP 1 020 197 A (FLEISCHMANN WILHELM DR MED) 19 July 2000 (2000-07-19) abstract; claims 1,2 ---	1,4, 10-13,17
X	WO 01 31033 A (PRITCHARD DAVID IDRIS ;UNIV NOTTINGHAM (GB)) 3 May 2001 (2001-05-03) page 5; claims 6,13 ---	11-13, 16,17
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☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the International filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the International filing date but later than the priority date claimed

- *T* later document published after the International filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *G* document member of the same patent family

Date of the actual completion of the International search

7 October 2003

Date of mailing of the International search report

16/10/2003

Name and mailing address of the ISA

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Authorized officer

Gonzalez Ramon, N

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 03/00959

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 1,4-7 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☒ Claims Nos.: 1-17 partially
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1-17 partially

Present claims 1-17 relate to compounds defined by reference to desirable characteristics or properties, namely: "substance having N-acyl homoserine lactone degradant activity" (claims 1, 10), "antibiotic" (claims 6, 8, 14); "a serine proteinase" (claim 11); "a glycosidase" (claim 12); "a substance having cecropin-like activity" (claim 13). Furthermore claims 10-13, 16 relate to an extremely large number of possible compounds defined as "analogues" (claims 10-13) or "synthetic analogue" (claim 16). In fact, the claims contain so many options and possible variables that a lack of clarity (and conciseness) within the meaning of Article 6 PCT arises to such an extent as to render a meaningful search of the claims impossible.

The claims cover all compounds having these characteristics or properties, whereas the application provides support within the meaning of Article 6 PCT and disclosure within the meaning of Article 5 PCT for only a very limited number of such compounds. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the compounds by reference to a result to be achieved: "substance having N-acyl homoserine lactone degradant activity" (claims 1, 10), "antibiotic" (claims 6, 8, 14); "a serine proteinase" (claim 11); "a glycosidase" (claim 12); "a substance having cecropin-like activity" (claim 13). Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to the compounds specifically mentioned by chemical name in the examples and those mentioned in claims 7, 9, 15 with due regard to the general idea underlying the present application.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Internat. Application No.

PC1 03/00959

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SIMMONS S. W.: "A bactericidal principle in excretions of surgical maggots which destroys important etiological agents of pyogenic infections" J BACTERIOL, vol. 30, 1935, pages 253-267, XP008019840 page 257 page 266, paragraph 3 page 254, paragraphs 2,3	1,4,5, 10-13,16
Y	----- TSUJI Y. ET AL: "Antibacterial activity of a novel 26kDa serine protease in the yellow body of Sarcophaga peregrina (flesh fly) pupae" FEBS LETTERS, vol. 425, 1998, pages 131-133, XP002248316 table 1 see discussion	1-17
A	----- FRIEDMAN E. ET AL: "Partially purified antibacterial agent from maggots displays a wide range of antibacterial activity" THIRD INTERNATIONAL CONFERENCE ON BIOTHERAPY, May 1998 (1998-05), XP001161249 Jerusalem, Israel abstract	1-17
Y	----- THOMAS S. ET AL: "The antimicrobial activity of maggot secretions: results of a preliminary study" J. TISSUE VIABILITY, vol. 9, no. 4, 1999, pages 127-132, XP008019698 page 128; figures 2-4,7	1,4,5, 10-13,16
X	----- CHAMBERS L ET AL: "DEGRADATION OF EXTRACELLULAR MATRIX COMPONENTS BY DEFINED PROTEINASES FROM THE GREENBOTTLE LARVA LUCILIA SERICATA USED FOR THE CLINICAL DEBRIDEMENT OF NON-HEALING WOUNDS" BRITISH JOURNAL OF DERMATOLOGY, XX, XX, vol. 148, no. 1, January 2003 (2003-01), pages 14-23, XP001150327 ISSN: 0007-0963 abstract see discussion	11-13, 16,17
X,P	----- MASERITZ I. H.: "Digestion of bone by larvae of phormia regina" ARCH. SURG, vol. 28, 1934, pages 589-607, XP008021206 page 599; table 6	1-17
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INTERNATIONAL SEARCH REPORT

International Application No
PCT/JP03/00959

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			EP 1222285 A2	17-07-2002
			WO 0131033 A2	03-05-2001